

WHAT IS CLAIMED IS:

1. A process for producing a sugar nucleotide, which comprises:

selecting, as enzyme sources, a) a culture broth of a microorganism capable of producing nucleotide-5'-triphosphate (referred to as "NTP" hereinafter) from a nucleotide precursor, or a treated product of the culture broth, and b) a culture broth of a microorganism capable of producing a sugar nucleotide from a sugar and NTP, or a treated product of the culture broth;

allowing the enzyme sources, the nucleotide precursor and the sugar to be present in an aqueous medium to form and accumulate the sugar nucleotide in the aqueous medium; and

recovering the sugar nucleotide from the aqueous medium.

2. A process for producing a complex carbohydrate, which comprises:

selecting, as enzyme sources, a) a culture broth of a microorganism capable of producing nucleotide-5'-triphosphate (referred to as "NTP" hereinafter) from a nucleotide precursor, or a treated product of the culture broth, b) a culture broth of a microorganism capable of producing a sugar nucleotide from a sugar and NTP, or a treated product of the culture broth, and c) a culture broth of a microorganism, an animal cell or an insect cell capable of producing a complex

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carbohydrate from a sugar nucleotide and a complex carbohydrate precursor, or a treated product of the culture broth;

allowing the enzyme sources, the nucleotide precursor, the sugar and the complex carbohydrate precursor to be present in an aqueous medium to form and accumulate the complex carbohydrate in the aqueous medium; and

recovering the complex carbohydrate from the aqueous medium.

3. A process for producing a complex carbohydrate, which comprises:

selecting, as an enzyme source, a culture broth of a microorganism, an animal cell or an insect cell capable of producing a complex carbohydrate from a sugar nucleotide and a complex carbohydrate precursor, or a treated product of the culture broth;

allowing the enzyme source, the complex carbohydrate precursor and the sugar nucleotide prepared by the process of claim 1 to be present in an aqueous medium to form and accumulate the complex carbohydrate in the aqueous medium; and

recovering the complex carbohydrate from the aqueous medium.

4. The process according to any one of claims 1, 2 and 3, wherein the treated product of culture broth is a

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concentrated product of the culture broth, a dried product of the culture broth, a culture supernatant obtained by centrifuging the culture broth, a concentrated product of the culture supernatant, an enzyme preparation obtained from the culture supernatant, cells obtained by centrifuging the culture broth, a dried product of the cells, a freeze-dried product of the cells, a surfactant-treated product of the cells, an ultrasonic-treated product of the cells, a mechanically disrupted product of the cells, a solvent-treated product of the cells, an enzyme-treated product of the cells, a protein fraction of the cells, an immobilized product of the cells or an enzyme preparation obtained by extraction from the cells.

5. The process according to claim 1 or 2, wherein the nucleotide precursor is orotic acid, uracil, orotidine, uridine, cytosine, cytidine, adenine, adenosine, guanine, guanosine, hypoxanthine, inosine, xanthine, xanthosine, inosine-5'-monophosphate, xanthosine-5'-monophosphate, guanosine-5'-monophosphate, uridine-5'-monophosphate or cytidine-5'-monophosphate.

6. The process according to any one of claims 1, 2 and 3, wherein the sugar nucleotide is a uridine diphosphate compound, a guanosine diphosphate compound or a cytidine monophosphate compound.

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7. The process according to claim 6, wherein the uridine diphosphate compound, guanosine diphosphate compound or cytidine monophosphate compound is a compound selected from uridine diphosphoglucose, uridine diphosphogalactose, uridine diphospho-N-acetylglucosamine, uridine diphospho-N-acetylgalactosamine, uridine diphosphoglucuronic acid, guanosine diphosphomannose, guanosine diphosphofucose, cytidine monophospho-N-acetylneuraminic acid, and derivatives thereof.

8. The process according to claim 1 or 2, wherein the sugar is a sugar selected from glucose, fructose, galactose, glucosamine, N-acetylglucosamine, N-acetylgalactosamine, mannose, fucose, N-acetylmannosamine, acetylneuraminic acid, and derivatives thereof.

9. The process according to claim 2 or 3, wherein the complex carbohydrate is a complex carbohydrate which contains at least one of sugars selected from glucose, galactose, N-acetylglucosamine, N-acetylgalactosamine, glucuronic acid, mannose, N-acetylmannosamine, fucose, sialic acid, lactose, N-acetyllactosamine, lacto-N-biose, GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc, GlcNAc $\beta$ 1-4Gal $\beta$ 1-4Glc, globotriose, Gal $\alpha$ 1-4Gal $\beta$ 1-4GlcNAc, 2'-fucosyllactose, 3-fucosyllactose, 3'-sialyllactose, 6'-sialyllactose, 3'-sialyl-N-acetyllactosamine, sialyllacto-N-biose, Lewis X, Lewis a, lacto-N-tetraose,

lacto-N-neotetraose, lactodifucotetraose, 3'-sialyl-3-fucosyllactose, sialyl-Lewis X, sialyl-Lewis a, lacto-N-fucopentaose I, lacto-N-fucopentaose II, lacto-N-fucopentaose III, lacto-N-fucopentaose V, LS-tetrasaccharide a, LS-tetrasaccharide b, LS-tetrasaccharide c, a  
a (α2,3)sialyllacto-N-neotetraose, lacto-N-difucohexaose I, lacto-N-difucohexaose II, lacto-N-hexaose, lacto-N-neohexaose, disialyllacto-N-tetraose, and derivatives thereof, or a complex carbohydrate which contains the complex carbohydrate.

10. The process according to claim 2 or 3, wherein the complex carbohydrate is a complex carbohydrate which contains a sugar having a bond selected from Gal $\beta$ 1-3Glc, Gal $\beta$ 1-4Glc, Gal $\beta$ 1-3GlcNAc, Gal $\beta$ 1-4GlcNAc, Gal $\beta$ 1-3Gal, Gal $\beta$ 1-4Gal, Gal $\beta$ 1-3GalNAc, Gal $\beta$ 1-4GalNAc, Gal $\alpha$ 1-3Glc, Gal $\alpha$ 1-4Glc, Gal $\alpha$ 1-3GalNAc, Gal $\alpha$ 1-4GalNAc, GlcNAc $\beta$ 1-3Gal, GlcNAc $\beta$ 1-4Gal, GlcNAc $\beta$ 1-6Gal, GlcNAc $\beta$ 1-3Glc, GlcNAc $\beta$ 1-4Glc, GlcNAc $\beta$ 1-3GlcNAc, GlcNAc $\beta$ 1-4GlcNAc, GlcNAc $\beta$ 1-6GalNAc, GlcNAc $\beta$ 1-2Man, GlcNAc $\beta$ 1-4Man, GlcNAc $\beta$ 1-6Man, GalNAc $\beta$ 1-3Gal, GalNAc $\beta$ 1-4Gal, GalNAc $\beta$ 1-4GlcNAc, GalNAc $\beta$ 1-3GalNAc, Man $\beta$ 1-4GlcNAc, Man $\alpha$ 1-6Man, Man $\alpha$ 1-3Man, Man $\alpha$ 1-2Man, GlcUA $\beta$ 1-4GlcN, GlcUA $\beta$ 1-3Gal, GlcUA $\beta$ 1-3GlcNAc, GlcUA $\beta$ 1-3GalNAc, NeuAco2-3Gal, NeuAco2-6Gal, NeuAco2-3GlcNAc, NeuAco2-6GlcNAc, NeuAco2-3GalNAc, NeuAco2-6GalNAc, NeuAco2-8NeuAc, Fuc $\alpha$ 1-3Glc, Fuc $\alpha$ 1-4Glc, Fuc $\alpha$ 1-3GlcNAc, Fuc $\alpha$ 1-4GlcNAc, Fuc $\alpha$ 1-2Gal and

Fuc $\alpha$ 1-6GlcNAc, or a complex carbohydrate which contains the complex carbohydrate.

a 11. The process according to claim 9 or 10, wherein the number of sugars contained in the complex carbohydrate is 10 or below.

a 12. The process according to claim 9 or 10, wherein the number of sugars contained in the complex carbohydrate is 6 or below.

13. The process according to claim 2 or 3, wherein the complex carbohydrate precursor is a complex carbohydrate precursor selected from monosaccharides, oligosaccharides, proteins, peptides, lipids, glycoproteins, glycolipids, glycopeptides and steroid compounds.

14. The process according to claim 13, wherein the complex carbohydrate precursor is a complex carbohydrate precursor selected from glucose, galactose, mannose, sialic acid, N-acetylglucosamine, N-acetylgalactosamine, lactose, N-acetyllactosamine, lacto-N-biose, GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc, GlcNAc $\beta$ 1-4Gal $\beta$ 1-4Glc, globotriose, Gal $\alpha$ 1-4Gal $\beta$ 1-4GlcNAc, 2'-fucosyllactose, 3-fucosyllactose, 3'-sialyllactose, 6'-sialyllactose, 3'-sialyl-N-acetyllactosamine, 6'-sialyl-N-acetyllactosamine, sialyllacto-N-biose, Lewis X, Lewis a, lacto-N-tetraose, lacto-N-neotetraose, lactodifucotetraose, 3'-sialyl-3-fucosyllactose, sialyl-Lewis X, sialyl-Lewis a, lacto-N-fucopentaose I, lacto-N-fucopentaose II, lacto-N-

fucopentaose III, lacto-N-fucopentaose V, LS-tetrasaccharide a, LS-tetrasaccharide b, LS-tetrasaccharide c, (α2,3)sialyllacto-N-neotetraose, and derivatives thereof, serine, threonine, asparagine and peptides containing these amino acids and derivatives thereof, and ceramide and derivatives thereof, or a complex carbohydrate precursor containing the complex carbohydrate precursor.

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15. The process according to claim 1 or 2, wherein the microorganism capable of producing NTP from a nucleotide precursor is a microorganism selected from microorganisms belonging to the genus *Corynebacterium*.

16. The process according to claim 15, wherein the microorganism belonging to the genus *Corynebacterium* belongs to *Corynebacterium ammoniagenes*.

17. The process according to claim 1 or 2, wherein the microorganism capable of producing a sugar nucleotide from a sugar and NTP is carried by comprises at least one kind of microorganism.

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18. The process according to claim 17, wherein at least one microorganism is selected from microorganisms belonging to the genus *Escherichia* and the genus *Corynebacterium*.

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19. The process according to claim 18, wherein the microorganism belonging to the genus *Escherichia* is *Escherichia coli*.

20. The process according to claim 18, wherein the microorganism belonging to the genus *Corynebacterium* is *Corynebacterium ammoniagenes*.

21. The process according to claim 1 or 2, wherein the microorganism capable of producing a sugar nucleotide from a sugar and NTP is a microorganism having strong activity of at least one enzyme selected from glucokinase (referred to as "glk" hereinafter), phosphoglucomutase (referred to as "pgm" hereinafter), glucose-1-phosphate uridylyltransferase (referred to as "galU" hereinafter) and pyrophosphatase (referred to as "ppa" hereinafter).

22. The process according to claim 21, wherein the microorganism comprises at least one microorganism having a recombinant DNA comprising a vector and a DNA fragment which contains at least one gene selected from a glk-encoding gene, a pgm-encoding gene, a galU-encoding gene and a ppa-encoding gene.

23. The process according to claim 22, wherein the glk-encoding gene, the pgm-encoding gene, the galU-encoding gene and the ppa-encoding gene are genes derived from *Escherichia coli*.

24. The process according to claim 21, wherein the sugar nucleotide is uridine diphosphoglucose.

25. The process according to claim 21, wherein the microorganism is a microorganism having strong uridine

diphosphoglucose dehydrogenase activity, and the sugar nucleotide is uridine diphosphoglucuronic acid.

26. The process according to claim 1 or 2, wherein the microorganism capable of producing a sugar nucleotide from a sugar and NTP is a microorganism having strong galactokinase (referred to as "galK" hereinafter) activity.

27. The process according to claim 26, wherein N-acetylglucosamine-1-phosphate is provided by the microorganism of claim 26 having strong galK activity using N-acetylglucosamine as a substrate.

28. The process according to claim 26, wherein the microorganism comprises at least one microorganism having a recombinant DNA comprising a vector and a DNA fragment which contains a galK-encoding gene. | a

29. The process according to claim 28, wherein the galK-encoding gene is a gene derived from *Escherichia coli*.

30. The process according to claim 26, wherein the microorganism capable of producing a sugar nucleotide from a sugar and NTP is a microorganism having strong galactose-1-phosphate uridyltransferase (referred to as "galT" hereinafter) activity.

31. The process according to claim 30, wherein the microorganism comprises at least one microorganism having a recombinant DNA comprising a vector and a DNA fragment which contains a galT-encoding gene. | a

32. The process according to claim 31, wherein the galT-encoding gene is a gene derived from *Escherichia coli*.

33. The process according to claim 30, wherein the microorganism capable of producing a sugar nucleotide from a sugar and NTP is a microorganism having strong activity of at least one enzyme selected from glucokinase (referred to as "glk" hereinafter), phosphoglucomutase (referred to as "pgm" hereinafter), glucose-1-phosphate uridylyltransferase (referred to as "galU" hereinafter) and pyrophosphatase (referred to as "ppa" hereinafter).

34. The process according to claim 33, wherein the microorganism comprises at least one microorganism having a recombinant DNA comprising a vector and a DNA fragment which contains at least one gene selected from a glk-encoding gene, a pgm-encoding gene, a galU-encoding gene and a ppa-encoding gene.

35. The process according to claim 34, wherein the glk-encoding gene, the pgm-encoding gene, the galU-encoding gene and the ppa-encoding gene are genes derived from *Escherichia coli*.

a 36. The process according to claim 30 or 33, wherein the sugar nucleotide is uridine diphosphogalactose.

37. The process according to claim 26, wherein the microorganism capable of producing a sugar nucleotide from a sugar and NTP is a microorganism having strong

N-acetylglucosamine-1-phosphate uridyltransferase (referred to as "glmU" hereinafter) activity.

38. The process according to claim 37, wherein the microorganism comprises at least one microorganism having a recombinant DNA comprising a vector and a DNA fragment which contains a glmU-encoding gene.

39. The process according to claim 38, wherein the glmU-encoding gene is a gene derived from *Escherichia coli*.

40. The process according to claim 1 or 2, wherein the microorganism capable of producing a sugar nucleotide from a sugar and NTP is a microorganism having strong phosphoglucomutase (referred to as "pgm" hereinafter) and phosphofructokinase (referred to as "pfkB" hereinafter) activities.

41. The process according to claim 40, wherein the microorganism comprises at least one microorganism having a recombinant DNA comprising a vector and a DNA fragment which contains at least one gene selected from a pgm-encoding gene and a pfkB-encoding gene.

42. The process according to claim 41, wherein the pgm-encoding gene and the pfkB-encoding gene are genes derived from *Escherichia coli*.

43. The process according to claim 40, wherein glucose-1,6-diphosphate is provided by the microorganism of

claim 40 having strong pgm and pfkB activities using glucose-6-phosphate and fructose-6-phosphate as a substrate.

44. The process according to claim 43, wherein phosphoglucosamine mutase or phosphomannomutase activity is increased by glucose-1,6-diphosphate provided by the process of claim 43.

45. The process according to claim 40, wherein the microorganism capable of producing a sugar nucleotide from a sugar and NTP is a microorganism having strong activity of at least one enzyme selected from glucosamine-1-phosphate acetyltransferase, N-acetylglucosamine-1-phosphate uridyltransferase (referred to as "glmU" hereinafter), pyrophosphatase (referred to as "ppa" hereinafter), phosphoglucosamine mutase (referred to as "glmM" hereinafter) and glucokinase (referred to as "glk" hereinafter).

46. The process according to claim 45, wherein the microorganism comprises at least one microorganism having a recombinant DNA comprising a vector and a DNA fragment which contains at least one gene selected from a glmU-encoding gene, a ppa-encoding gene, a glmM-encoding gene and a glk-encoding gene.

47. The process according to claim 46, wherein the glmU-encoding gene, the ppa-encoding gene, the glmM-encoding gene and the glk-encoding gene are genes derived from *Escherichia coli*.

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48. The process according to any one of claims 26, 37, 40 and 45, wherein the sugar nucleotide is uridine diphospho-N-acetylglucosamine.

49. The process according to any one of claims 26, 37, 40 and 45, wherein the microorganism is a microorganism having strong UDP-GlcNAc 4-epimerase activity and the sugar nucleotide is uridine diphospho-N-acetylgalactosamine.

50. The process according to claim 40, wherein the microorganism capable of producing a sugar nucleotide from a sugar and NTP is a microorganism having strong activity of at least one enzyme selected from phosphomannomutase (referred to as "manB" hereinafter), mannose-1-phosphate guanyltransferase (referred to as "manC" hereinafter) and glucokinase (referred to as "glk" hereinafter).

51. The process according to claim 50, wherein the microorganism comprises at least one microorganism having a recombinant DNA comprising a vector and a DNA fragment which contains at least one gene selected from a manB-encoding gene, a manC-encoding gene and a glk-encoding gene.

52. The process according to claim 51, wherein the manB-encoding gene, the manC-encoding gene and the glk-encoding gene are genes derived from *Escherichia coli*.

53. The process according to claim 40 or 50, wherein the sugar nucleotide is guanosine diphosphomannose.

54. The process according to claim 40, wherein the microorganism capable of producing a sugar nucleotide from a sugar and NTP is a microorganism having strong activity of at least one enzyme selected from phosphomannomutase (referred to as "manB" hereinafter), mannose-1-phosphate guanyltransferase (referred to as "manC" hereinafter), glucokinase (referred to as "glk" hereinafter), GDP-4,6-mannose dehydratase (referred to as "gmd" hereinafter) and GDP-4-keto-6-deoxymannose epimerase/reductase (referred to as "wcaG" hereinafter).

55. The process according to claim 54, wherein the microorganism comprises at least one microorganism having a recombinant DNA comprising a vector and a DNA fragment which contains at least one gene selected from a manB-encoding gene, a manC-encoding gene, a glk-encoding gene, a gmd-encoding gene and a wcaG-encoding gene.

56. The process according to claim 55, wherein the manB-encoding gene, the manC-encoding gene, the glk-encoding gene, the gmd-encoding gene and the wcaG-encoding gene are genes derived from *Escherichia coli*.

57. The process according to claim 51 or 54, wherein the sugar nucleotide is guanosine diphosphofucose.

58. The process according to claim 1 or 2, wherein the microorganism capable of producing a sugar nucleotide from a sugar and NTP is a microorganism having strong

activity of at least one enzyme selected from GlcNAc 2-epimerase, CMP-NeuAc synthetase (referred to as "neuA" hereinafter), NeuAc aldolase (referred to as "nanA" hereinafter), NeuAc synthetase (referred to as "neuB" hereinafter) and CTP synthetase (referred to as "pyrG" hereinafter).

59. The process according to claim 58, wherein the microorganism comprises at least one microorganism having a recombinant DNA comprising a vector and a DNA fragment which contains at least one gene selected from a GlcNAc 2-epimerase-encoding gene, a neuA-encoding gene, a nanA-encoding gene, a neuB-encoding gene and a pyrG-encoding gene.

60. The process according to claim 59, wherein the neuA-encoding gene, the nanA-encoding gene, the neuB-encoding gene and the pyrG-encoding gene are genes derived from *Escherichia coli*.

61. The process according to claim 58, wherein the sugar nucleotide is cytidine monophospho-N-acetylneurameric acid.

62. The process according to claim 2 or 3, wherein the microorganism capable of producing a complex carbohydrate from a sugar nucleotide and a complex carbohydrate precursor belongs to *Escherichia coli*, *Saccharomyces cerevisiae* or *Corynebacterium ammoniagenes*.